PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 04 September 2000 (04.09.00)	
International application No. PCT/US99/24017	**Applicant's or agent's file reference** F134222
International filing date (day/month/year) 12 November 1999 (12.11.99)	**Priority date** (day/month/year) 13 November 1998 (13.11.98)
Applicant BUKOVSKY, Anatoly et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

31 May 2000 (31.05.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nestor Santesso
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

09/831627

5060

16

PATENT COOPERATION TREATY

PCT

REC'D 15 MAY 2001

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference F134222	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US99/24017	International filing date *(day/month/year)* 12 NOVEMBER 1999	Priority date *(day/month/year)* 13 NOVEMBER 1998
International Patent Classification (IPC) or national classification and IPC IPC(7): C12N 7/00 and US Cl.: 435/235.1		
Applicant CELL GENESYS, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of _____ sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☐ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
31 MAY 2000	24 APRIL 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT A. ZEMAN Telephone No. (703) 308-0196

TERRY J. DEY
PARALEGAL SPECIALIST
TECHNOLOGY CENTER 1600

Form PCT/IPEA/409 (cover sheet) (July 1998) ★

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/24017

I. Basis of the report

1. With regard to the **elements** of the international application: *

[X] the international application as originally filed

[X] the description:
pages 1-9, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 10, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages 1-3, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[ ] contained in the international application in printed form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No.
PCT/US99/24017

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	NONE	YES
	Claims	1-3	NO
Inventive Step (IS)	Claims	NONE	YES
	Claims	1-3	NO
Industrial Applicability (IA)	Claims	1-3	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claims 1-3 lack novelty under PCT Article 33(2) as being anticipated by Gruber et al. (U.S. Patent 5,503,974).

Claims 1-3 are drawn a method of ampifying an envelope-defective retrovirus by exposing said retrovirus to a cell which expresses the viral envelope. Gruber et al. disclose the use of a defective retroviral vector which may contain *gag*, *pol* or *env* (see column 4, lines 33-37) for the detection of retroviruses. Additionally, Gruber et al. disclose that were primary cells are used it is preferable that the primary cells be given the ability to expresses viral packaging proteins (env proteins). The disclosure of Gruber et al. contains all the limitations of the instant claims. Specifically, the production of retroviruses through the utilization of "complementations i.e the production of the *env* proteins by a transfected cell to complement the incomplete viral replication cycle.

Claims 1-3 lack an inventive step under PCT Article 33(3) as being obvious over Gruber et al (U.S. Patent 5,503,974).

Claims 1-3 are drawn a method of ampifying an envelope-defective retrovirus by exposing said retrovirus to a cell which expresses the viral envelope. Gruber et al. disclose the use of a defective retroviral vector which may contain *gag*, *pol* or *env* (see column 4, lines 33-37) for the detection of retroviruses. Additionally, Gruber et al. disclose that were primary cells are used it is preferable that the primary cells be given the ability to expresses viral packaging proteins (env proteins). The disclosure of Gruber et al. contains all the limitations of the instant claims. Specifically, the production of retroviruses through the utilization of "complementations i.e the production of the *env* proteins by a transfected cell to complement the incomplete viral replication cycle.

---------- NEW CITATIONS ----------
NONE

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

Claim 2 is objected to under PCT Rule 66.2(a)(v) as lacking clarity under PCT Article 6 because the claim 2 is indefinite for the following reason(s): the phrase "wherein said virus envelope is expressed at the surface of a virus particle produced by said cell" is nonsensicle. In the retroviral replication cycle the envelope proteins are expressed on the surface of the infected cell and are aquired by the retroviruse by "budding" through the cellular membrane. It is unclear what the Applicant is claiming as the metes and bounds of said invention.

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
NAKAMURA, Dean, H.
Sughrue, Mion, Zinn, MacPeak & Seas, PLLC
Suite 800
2100 Pennsylvania Avenue, N.W.
Washington, DC 20037-3202
ETATS-UNIS D'AMERIQUE

Date of mailing (day/month/year) 25 May 2000 (25.05.00)	
Applicant's or agent's file reference F134222	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/US99/24017	12 November 1999 (12.11.99)	13 November 1998 (13.11.98)

Applicant
CELL GENESYS, INC. et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,MA,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
   GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,
   PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   25 May 2000 (25.05.00) under No. WO 00/29557

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer
J. Zahra

Telephone No. (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24017

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : C12N 7/00
US CL : 435/235.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/235.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,503,974 A (GRUBER ET AL) 02 April 1996 (02/04/96), see entire document, especially columns 4 and 6.	1-3
Y	US 5,591,579 A (OLIVO ET AL) 07 January 1997 (07/01/97), see entire document.	1-3
Y	US 5,614,404 A (MAZZARA ETAL) 25 March 1997 (25/03/97), see entire document.	1-3
Y	US 5,583,022 A (HEIDMANN ET AL) 10 December 1996 (10/12/96), see entire document.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 08 FEBRUARY 2000	Date of mailing of the international search report 23 FEB 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer ROBERT A. ZEMAN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

International application No.
PCT/US99/24017

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST, Medline, CAPlus, Biosis.

search terms: defective, retrovirus, amplification, detection, cell, indicator, envelope, env

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:
DEAN H. NAKAMURA
SUGHRUE, MION, ZINN, MACPEAK &
SEAS, PLLC
2100 PENNSYLVANIA AVE., N.W., STE. 800
WASHINGTON, DC 20037 3202

PCT

NOTIFICATION OF RECEIPT OF DEMAND BY COMPETENT INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

(PCT Rules 59.3(e) and 61.1(b), first sentence and Administrative Instructions, Section 601(a))

Date of mailing *(day/month/year)*	26 JUL 2000
Applicant's or agent's file reference: F134222	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US99/24017	12 NOV 99	13 NOV 98

Applicant
CELL GENESYS, INC.

1. The applicant is hereby **notified** that this International Preliminary Examining Authority considers the following date as the date of receipt of the demand for international preliminary examination of the international application:

   31 MAY 2000 (31.05.00)

2. That date of receipt is:

   [X] the actual date of receipt of the demand by this Authority (Rule 61.1(b)).

   [ ] the actual date of receipt of the demand on behalf of this Authority (Rule 59.3(e)).

   [ ] the date on which this Authority has, in response to the invitation to correct defects in the demand (Form PCT/IPEA/404), received the required corrections.

3. [ ] **ATTENTION:** That date of receipt is **AFTER** the expiration of 19 months from the priority date. Consequently, the election(s) made in the demand does (do) not have the effect of postponing the entry into the national phase until 30 months from the priority date (or later in some Offices) (Article 39(1)). Therefore, the acts for entry into the national phase must be performed within 20 months from the priority date (or later in some Offices) (Article 22). For details, see the *PCT Applicant's Guide*, Volume II.

   [ ] *(If applicable)* This notification confirms the information given by telephone, facsimile transmission or in person on:

   _____

4. Only where paragraph 3 applies, a copy of this notification has been sent to the International Bureau.

Name and mailing address of the IPEA/	Authorized officer
Assistant Commissioner for Patent Box PCT Washington, D.C. 20231 Attn:RO/US Facsimile No. 703-305-3230	Felicia Lawrence PCT Operations - TAPD Team 1 Telephone No. (703) 305-3675 (703) 305-3230 (FAX)

Form PCT/IPEA/402 (July 1998)

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 7/00

A1

(11) International Publication Number: WO 00/29557

(43) International Publication Date: 25 May 2000 (25.05.00)

(21) International Application Number: PCT/US99/24017

(22) International Filing Date: 12 November 1999 (12.11.99)

(30) Priority Data:
60/108,168 13 November 1998 (13.11.98) US

(71) Applicant *(for all designated States except US)*: CELL GENESYS, INC. [US/US]; 342 Lakeside Drive, Foster City, CA 94404 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: BUKOVSKY, Anatoly [RU/US]; Cell Genesys, Inc., 342 Lakeside Drive, Foster City, CA 94404 (US). NALDINI, Luigi [IT/US]; Cell Genesys, Inc., 342 Lakeside Drive, Foster City, CA 94404 (US).

(74) Agents: NAKAMURA, Dean, H. et al.; Sughrue, Mion, Zinn, MacPeak & Seas, PLLC, Suite 800, 2100 Pennsylvania Avenue, N.W., Washington, DC 20037–3202 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: A SENSITIVE SCREENING SYSTEM FOR ENVELOPE–DEFECTIVE RECOMBINANT VIRUS

(57) Abstract

An indicator cell line which transcomplements envelope defective recombinant virus can be used to amplify that virus.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

A Sensitive Screening System For Envelope-Defective Recombinant Virus

FIELD OF INVENTION

A sensitive screening system identifies envelope-defective recombinant viruses originating during production of lentiviral or retroviral vectors.

BACKGROUND OF THE INVENTION

Generally, recombinant viruses are replication-defective. However, such recombinant viruses still may be harmful to vector production in several ways. First, recombinant viruses may be propagated in vector producer cells. Second, recombinant viruses can interfere with the transduction of the vector by competing during encapsidation of the viral particles. Moreover, recombinant viruses may be harmful to a vector recipient due to the transfer of vector packaging functions. That may cause toxicity or an immune reaction in the transduced cells and host. There also may be an increase in the risk of additional recombination events which eventually could lead to generation of replication-competent retrovirus.

Such recombinant viruses may originate from the recombination of two or more of the constructs used to produce the vector, or from one such construct and endogenous retroviral sequences expressed in vector producer or target cells. Recombinants generally contain viral cis-acting sequences required for encapsidation and transfer to the target cells. Recombinants also generally contain the gag/pol gene sequences of a retrovirus or lentivirus.

The extent to which defective recombinants, such as envelope defective recombinants, contaminate batches of vector produced for clinical use or influence the performance of a vector producer system, is often unknown. The risk of defective versus replication competent recombinants occurring is

increased with the new split-genome packaging cell lines for retroviral vectors and with the use of vector pseudotyping due to the lack of any overlap between the constructs encoding the envelope and the gag/pol genes. Although that implies a lower risk of replication-competent recombinants occurring, the recombination between the gag/pol construct and the transfer vector carrying the foreign gene of interest may still occur and generate envelope-defective recombinants that go unnoticed in conventional screening.

Defective recombinants contaminating vector lots used in clinical trials also may be responsible for false positive results in certain assays used to monitor replication-competent recombinants in the recipients.

Sensitive detection and early elimination of defective recombinants thus is crucial to validate and to maintain the performance of a vector producer system, as well as to prove the purity and safety of a vector batch. However, as defective recombinants are replication-defective as well, routine assays used to monitor retroviral recombinants that are based on amplification through replication in the indicator cell line(s) cannot detect the defective recombinants.

SUMMARY OF THE INVENTION

The invention describes an amplification method which detects replication-defective recombinants that is based on transcomplementation in an indicator cell line which provides the missing packaging functions, for example, an envelope gene to detect envelope-defective recombinants. An important feature of the complementing envelope is little if no interference with superinfection of the indicator cells thereby allowing under certain circumstances amplification by pseudoreplication of the recombinant in a homogenous culture.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts how an envelope-defective recombinant can be generated from a packaging plasmid and a transfer vector carrying HIV sequences. The upper diagram depicts the wild-type HIV-1 genome. In the diagrams, LTR is a long terminal repeat; SD is a splice donor site; GAG is the group antigen coding sequence; PRO is the protease coding sequence; POL is the polymerase coding sequence; VIF and NEF are accessory genes; CMV is the cytomegalovirus enhancer/promoter; poly A is a polyadenylation site; the delta ENV designation indicated deletion of the envelope coding sequence; SA is a splice acceptor site; prom is a promoter; Transgene is a foreign gene of interest; ψ is an encapsidation signal sequence; TAT and REV are regulatory genes; and RRE is Rev Responsive Element. The solid blocks indicate the HIV open reading frames or functional genes in the three reading frames.

Figure 2 depicts the results of assays aimed at determining the sensitivity of the method of interest.

Figure 3 depicts the results of viral amplification in the absence or presence of the VSV G envelope.

DETAILED DESCRIPTION OF THE INVENTION

The detection of replication defective retroviral recombinants, particularly of lentivirus-based vectors, rests with providing in trans a complementing function for that suspected of being defective in the recombinants. For example, if detection of envelope-defective recombinants is desired, a means for providing envelope protein is utilized. That can be accomplished by developing transcomplementing cell lines which express one or more components needed for producing virus particles, such as an envelope protein.

An indicator cell line expressing a transcomplementing protein, such as, envelope protein, tat protein, rev protein or a combination thereof may be constructed and used as a screening agent in the instant invention.

Preferably the indicator cell lines are stable, transformed cells that express the one or more transcomplementing factors. Generally a stable, transformed cell in one wherein the transgene encoding the desirable factor is integrated into the host genome. That can be accomplished, for example, by transfection or by using a vector known to integrate into a host genome, such as, a retroviral vector, a transposon-based vector or an adeno-associated viral vector to carry the transgene.

Moreover, because a foreign gene product, such as an envelope protein, may be toxic to the host cell, it is desirable to regulate the expression of the transgene. For example, an inducible promoter may be used to control the expression of the transgene. Such inducible promoters are known in the art.

Otherwise, the making and maintenance of the transformed cell as well as the vectors of interest are as known in the art using materials readily available to the artisan. Any of a variety of host cells can be used. Moreover, the vectors and transgenes are known and the artisan can rely on known methods to construct a vector of interest.

Essentially, any known vector carrying a transcomplementing gene of interest and any suitable host cell can be used. Known inducible regulation systems can be used to regulate the expression of the transcomplementing gene. Also, any known method for detecting virus or expression of a gene product originating for the defective recombinant can be used, such as an immunoassay for a gag protein.

The 293G cell line which expresses the envelope protein G of vesicular stomatitis virus (VSV) (Ory et al., Proc. Natl. Acad. Sci. 93:11400-11406, 1996) can be used to detect envelope-defective viruses wherein the entry thereof

in target cells can be mediated by said protein G. The G protein is of interest because that envelope glycoprotein has been found to complement a wide range of viruses. Expression of the VSV G protein in 293G is controlled in a tetracycline regulated manner.

The VSV G cell line can be used, for example, to amplify minimal amounts of envelope-defective recombinant viruses that can nevertheless express and transfer the gag/pol genes of HIV. With that tetracycline regulated system, the cells are maintained in the presence of tetracycline which suppresses the expression of VSV G. Removal of tetracycline from the culture medium maintaining 293G cells results in induction of VSV G protein expression on the surface of the indicator cells thereby allowing for efficient pseudotyping and/or entry of the released viral particles. The particles in turn are capable of superinfecting the indicator cells which leads to amplification of the input viral recombinant.

The VSV G envelope is particularly useful by endowing the viral particles with a very high infectivity thereby enhancing the sensitivity and robustness of the assay.

Amplification of virus not only by viruses carrying the envelope glycoprotein but also by viruses which lack or do not express an envelope glycoprotein has been observed Thus, in the case of the VSV G protein, the expression of G protein at the surface of target cells is sufficient to mediate productive infection independent of expression within the viral membrane.

In view thereof, the instant invention relates to a method wherein screening of vector recipients following therapy can be accommodated. The method also can be used to amplify other enveloped viruses for which natural cellular receptors have yet to be identified.

The instant assay will find use, for example, in the production of efficient and safe HIV-based lentiviral vectors. The growth of the recombinant particles

can be detected by immunoenzymatic assays detecting, for example. the HIV p24 gag antigen, or by RNA-PCR assays for detecting the HIV gag gene. Then, the presence of defective recombinants can be monitored by the use of the instant indicator cells.

The instant method also can be used to identify partial recombinant viruses that express and transfer the gag/pol genes of other retroviruses, for example, the lentivirus, such as, the various SIV's, FIV, HIV-2, visna-maedi virus, caprine arthritis-encephalitis virus, BIV and equine infectious anemia virus, and other retrovirus, such as, spumavirus, murine leukemia and sarcoma viruses, other mammalian C-type viruses, such as FeLV and simian sarcoma virus, HERV's, B-type virus, such as mouse mammary tumor virus, D-type virus, HTLV's, bovine leukemia virus and avian leukosis-sarcoma viruses, such as Rous sarcoma virus and avian myeloblastosis virus. The detection system of the recombinant particle would be adjusted to the genes of the selected virus.

In another embodiment of the invention, the indicator cell line transcomplements one or both of the essential regulatory genes of lentivirus, tat and rev, in addition to the envelope gene. Such a system is useful to identify partial recombinants that contain the gag/pol genes of a lentivirus but do not express those genes efficiently, because for example, the regulatory genes required for efficient expression of the gag/pol genes are lacking or are defective. Suitable assays would be those detecting expression of gag or pol.

The amplification provided by the instant method in the case of detecting envelope-defective recombinants arises from the significantly more efficient viral entry mediated by the complementing envelope proteins as compared to the homologous or parental gene product. Thus, another use of the instant method is a fast selection of viral gene variants of a desired phenotype, such as drug-resistance or growth advantage. The selection could be performed without

the need of actually producing an infectious viral construct as a complementing envelope protein can be produced by a cell, such as an indicator cell of interest.

The invention now will be exemplified in the following non-limiting examples.

EXAMPLES

For validation of the detection system, an envelope-defective recombinant was constructed by molecular cloning using known techniques. A VSV G protein expressing construct, pMD.G, which does not contain HIV sequences, was used (Naldini et al., Science 272:263-267, 1996a). Viral particles were generated by co-transfecting an envelope-defective recombinant construct and the VSV.G expressing construct into 293T cells (Naldini et al., 1996a, supra; Proc. Natl. Acad. Sci. 93:11382-11388, 1996b). The recombinant construct was an HIV-based vector containing all but envelope sequences.

Control virus was produced by means of transient transfection of the envelope-defective recombinant plasmid R8.7 delE and the VSV.G expressor plasmid pMD G (Naldini et al., 1996a, supra) into 293T cells. The R8.7 plasmid was constructed by cloning the BclI-XhoI fragment of plasmid pCMVDR8.74 (Dull et al., J. Virol. 72:8463-8471, 1998), which contains the HIV gag, pol, tat and rev genes but no accessory genes, into R8 (Gallay et al., Cell 83:569-576, 1995).

Cells were seeded in 10 cm dishes 24 hours before infection and washed 2 hours before transfection. Culture medium (IMDM, 10% FCS) was replaced at 14 hours and transfectant-conditioned medium was collected at 36 hours post transfection. The conditioned medium was cleared by low-speed centrifugation (1500g) and passed through 0.45 μm filters. The amount of viral particles in the medium was measured by immunocapture assay for the HIV-1 p24 gag antigen (DuPont).

Titration of the viral particles on the VSV.G indicator cells by limiting dilution permitted an estimate of the sensitivity of the assay and provided a means to determine the amount of envelope-defective recombinants in vector preparations.

Indicator 293G cells (Ory et al., supra) and control 293-cells were seeded in 6-well plates at approximately 30% confluence 24 hours before infection. Immediately before infection, calls were washed with fresh medium. Control virus was diluted serially 10-fold in the medium without tetracycline and 1 ml of each dilution was added to each well. Cultured medium from infected cells were replaced regularly and amplification of recombinants was monitored by measuring p24 antigens in the supernatant. Infected cells were split 1/5 after confluence was reached.

As provided in Figure 2, the system is capable of detecting an inoculum of viral particles encapsidating an envelope-defective construct at a level of less than 20 fg p24 equivalent (lowest dilution used) in a 15 day incubation period. A gradual increase in p24 antigen concentration was observed in the culture supernatant on the indicated day after infection. No amplification of the recombinant was seen when 293 cells lacking the VSV envelope where infected with the same amount of viral particles.

The data of Figure 3 demonstrate the ability of the 293G cell to support virus amplification after initial infection by virus lacking envelope glycoprotein. Virions were produced by transient co-transfection of a plasmid encoding the HIV derivative containing deletion of the env gene and of either carrier DNA or the pMD.G plasmid encoding VSV G. Virions were normalized for p24 content, serially diluted (20, 0.5 and 0.125 ng/ml) and incubated with either induced 293/G cells or control 293 cells. As the expression of G in the 293G/ cell line is regulated by tetracycline, the cells were maintained in the absence of tetracycline 24 hours prior to infection.

During a two week period, supernatants of infected cells were monitored for p24 content as a measure of viral amplification. The data in Figure 3 are the results observed at the end of a two week period.

No p24 was detected in the supernatant of 293 cells incubated with envelope-defective virions.

As expected, 293 cells infected with VSV G pseudotyped viruses produced low levels of p24 which was proportional to the input amount of virus and was not amplified throughout the incubation period. On the other hand, 293G cells generated increasing amounts of p24 with identical kinetics whether pseudotyped or envelope defective virus was used for the initial infection.

We claim:

1. A method of amplifying an envelope-defective retrovirus by exposing said retrovirus to a cell comprising a virus envelope gene, wherein virus envelope encoded by said gene complements said retrovirus.

2. The method of claim 1, wherein said virus envelope is expressed at the surface of a virus particle produced by said cell.

3. The method of claim 1, wherein said virus envelope is expressed by said cell.

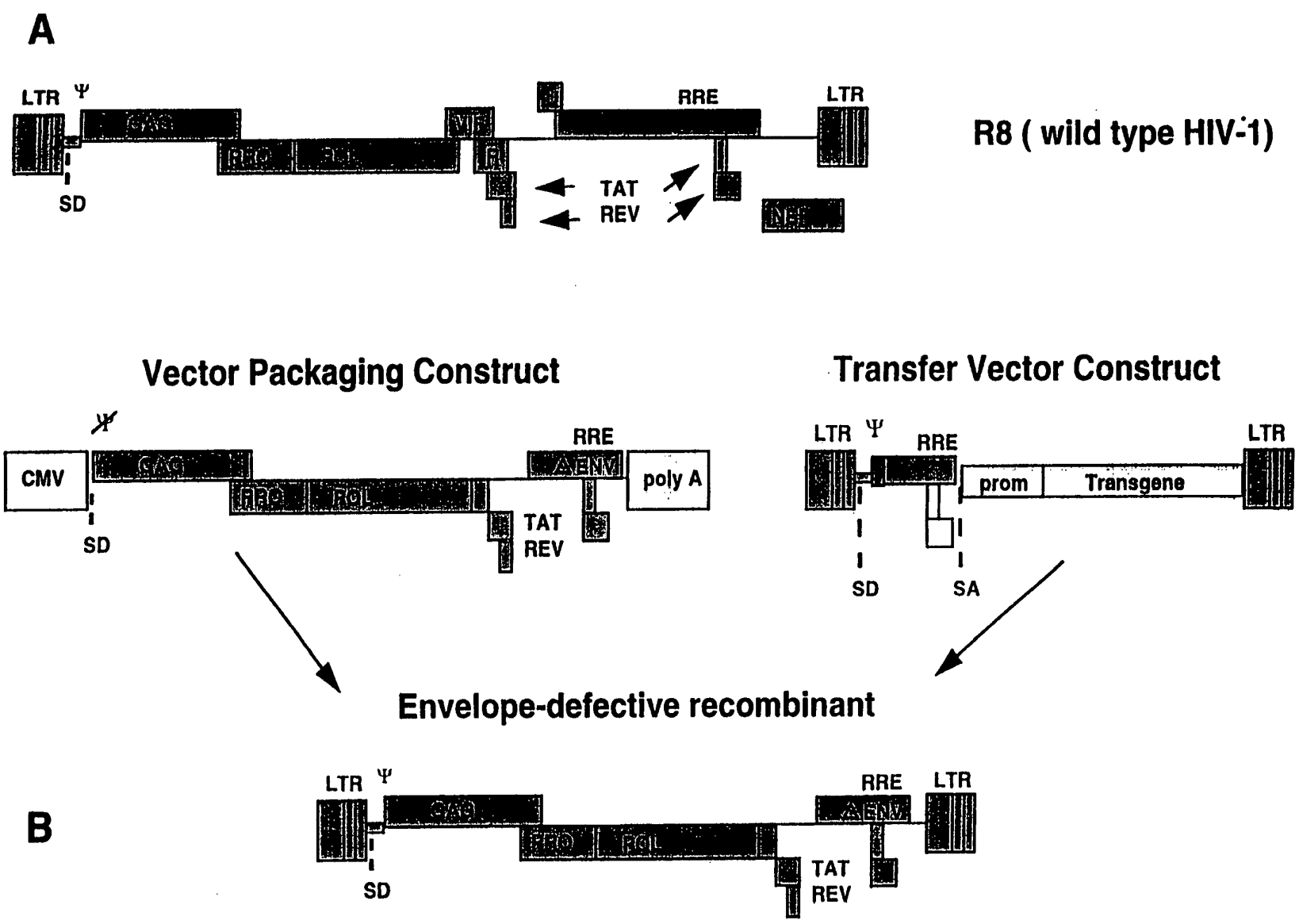
A
LTR
Ψ
GAG
PRO
POL
VIF
VPR
RRE
LTR
TAT
REV
SD
R8 (wild type HIV-1)
Vector Packaging Construct
CMV
GAG
PRO
POL
RRE
ΔENV
poly A
TAT
REV
SD
Transfer Vector Construct
LTR
Ψ
RRE
prom
Transgene
LTR
SD
SA
Envelope-defective recombinant
B
LTR
Ψ
GAG
PRO
POL
RRE
ΔENV
LTR
TAT
REV
SD

Figure 1

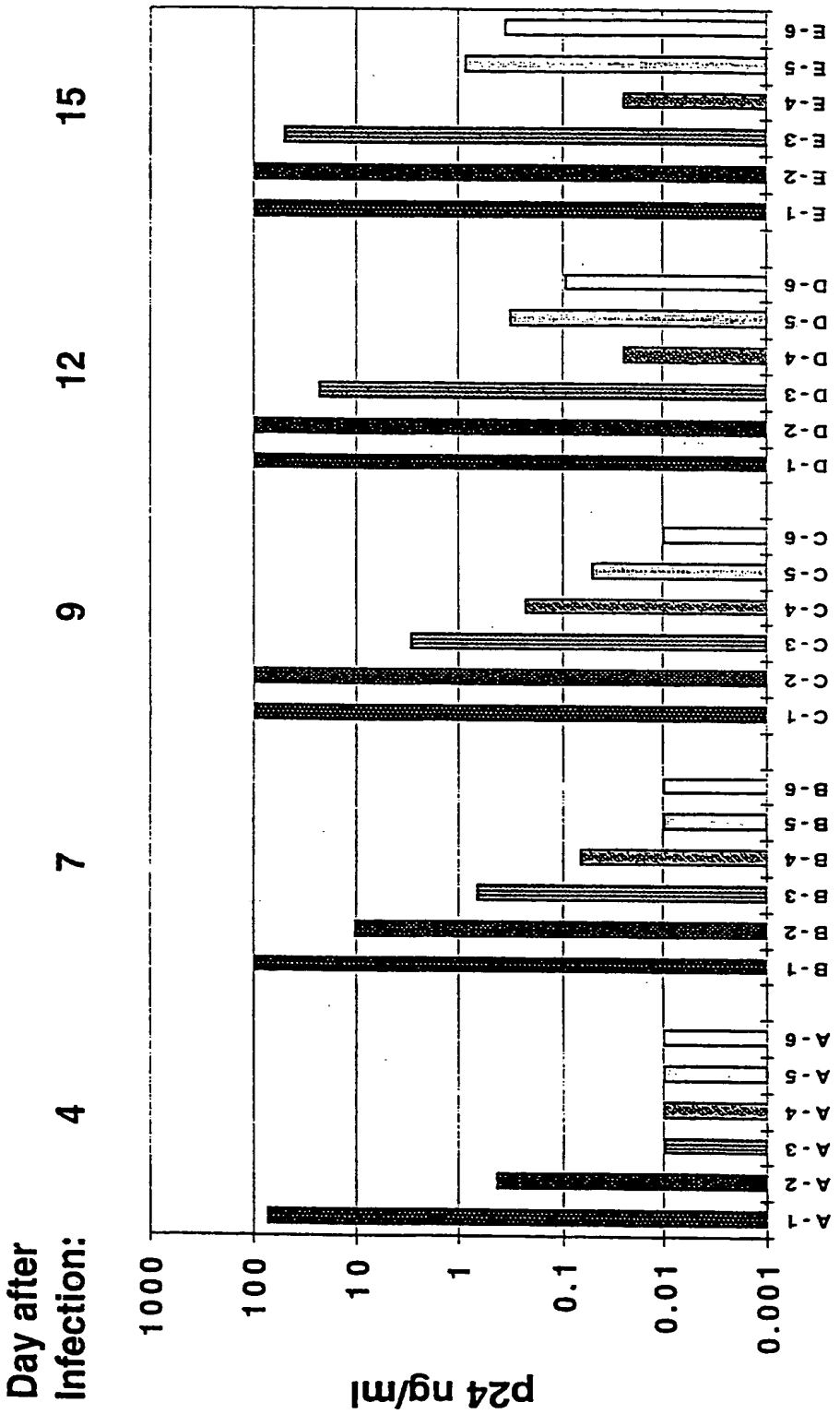

Figure 2. Amplification of partial recombinants lacking the Env gene.

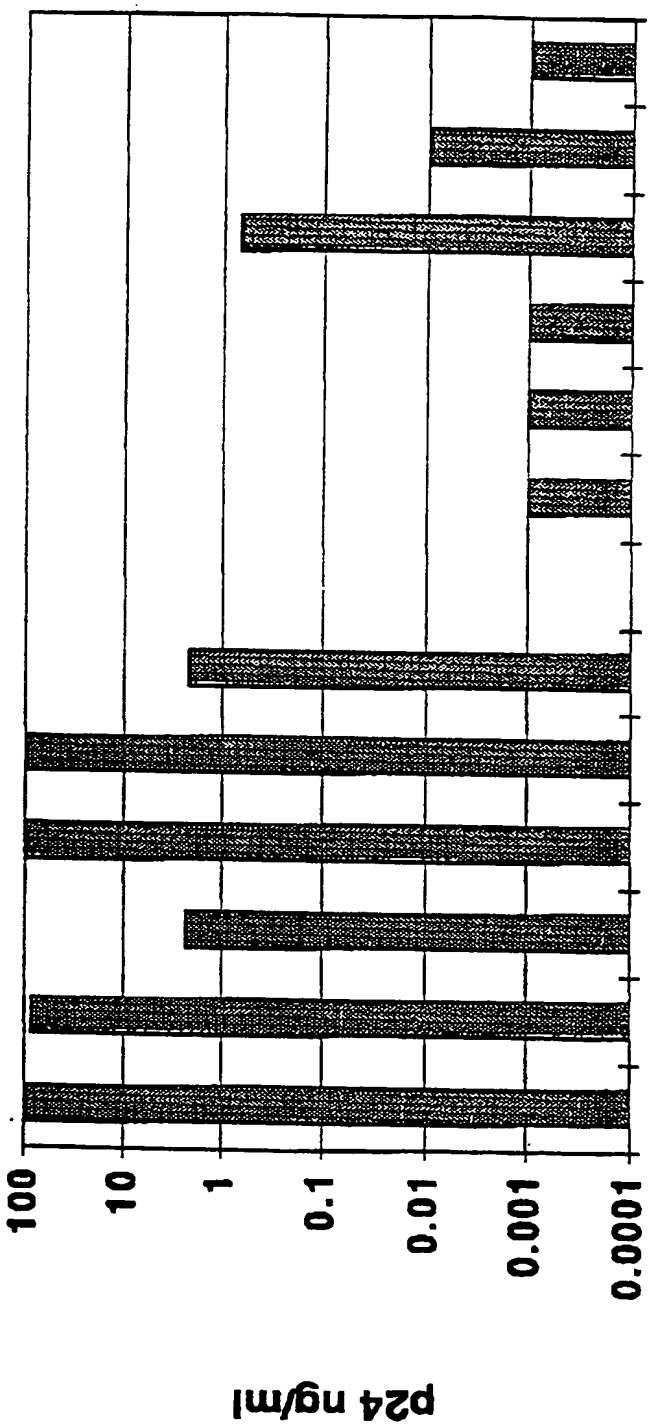

Figure 3 Efficient Amplification of virions lacking Env glycoprotein

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24017

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :C12N 7/00
US CL :435/235.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/235.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,503,974 A (GRUBER ET AL) 02 April 1996 (02/04/96), see entire document, especially columns 4 and 6.	1-3
Y	US 5,591,579 A (OLIVO ET AL) 07 January 1997 (07/01/97), see entire document.	1-3
Y	US 5,614,404 A (MAZZARA ETAL) 25 March 1997 (25/03/97), see entire document.	1-3
Y	US 5,583,022 A (HEIDMANN ET AL) 10 December 1996 (10/12/96), see entire document.	1-3

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
08 FEBRUARY 2000

Date of mailing of the international search report
23 FEB 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
ROBERT A. ZEMAN
Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/24017

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

WEST. Medline. CAPlus. Biosis.

search terms: defective. retrovirus. amplification. detection. cell. indicator. envelope. env